



Peptide conjugate construct - diagram - [modular structure]

For the detection of a cytoplasmic disulfide redox cleavage: each side of the disulfide construct carries a different fluorophor, as shown below; following the disulfide cleavage, the dyes are found separately in different compartments (cytoplasm; nucleus)

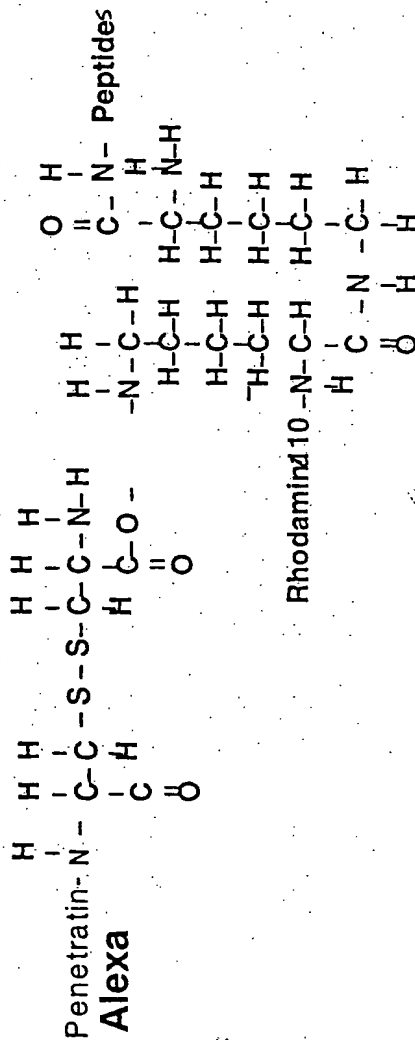
Module1: (MW 2517) **Alexa-Penetration-Cy-S-H** Module2: (MW 2597) **H-S-CyONH-NLS-NH-CH₂-(CH₂)₃-CH NH₂-CO-NH-CH₂-(CH₂)₃-CHNH₂-CO-NH-Peptide(Drug,PNA)**

-2H ↓ DMSO 20%/5h (coupling)/ purity: 90 - 95 % !!!!!

Alexa-Penetration-Cy-S-S-CyONH-NLS-NH-CH₂-(CH₂)₃-CHNH₂-CO-NH-CH₂-(CH₂)₃-CHNH₂-CO-NH-Peptide(Drug,PNA)

FITC	Transport	Redox cleavage	nuc. loc. sequ.
		lysine spacer	ε-NH-Rhodamin 10
			(Random-) model peptide

Penetratin: P: (pAntp): NH₂RQIKIWFQNRMRMKWKK-COOH



Penetratin: P: (pAntp): NLS: PNA_{AS}: PNA_{NS}:	transport protein (Nuc. Localis. Signal)	cystine redox cleavage site NH ₂ RQIKIWFQNRMRMKWKK-COOH NH ₂ PKKKRKV-COOH NH ₂ TAC TGC GAC TCC GG-COOH NH ₂ TTA AGG AGG CTC-COOH	nuclear localization sequence spacer model peptide
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Fig. 6



Time-dependent intracellular transport of the modules. (Z): cytoplasm; (N): nucleus;
 (+): positive signal; (-): no signal. Final conjugate concentration: 100 pM

Transporter	incubation period [h]	Z	N	method
Alexa™ ⁴⁸⁸ (L)-Penet-S-S-(L)-NLS-KK ^(Rhod110) -PNA	1	+	+	CLSM
	3	+	+	
	6	+	+	
	10	membrane spots	+	
	24	membrane spots	+	
Alexa™ ⁴⁸⁸ (L)-PTT ^(TAT/IIIIV-1) -S-S-(L)-KK ^(Rhod110) -PNA	1	+	-	CLSM
	3	+	-	
	10	+	-	
	24	-	-	
Alexa™ ⁴⁸⁸ (L)-TP ^(1A0P/EC0) -S-S-(L)-NLS-KK ^(Rhod110) -PNA	1	+	+	CLSM
	3	+	+	
	6	+	+	
	10	-	+	
	24	-	+	
Alexa™ ⁴⁸⁸ (L)-TP ^(1A0P/EC0) -S-S-(L)-KK ^(Rhod110) -PNA	1	+	-	CLSM
	3	+	-	
	6	+	-	
	10	-	-	
	24	-	-	

Fig. 8